Meat juice as an alternative sample to Hepatitis E virus ELISA: a pilot study in wild boar

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Abstract

Currently, the Hepatitis E virus (HEV) infection in the European wild boar population is considered an emerging Public Health issue. Constraints in obtaining a good blood sample from hunted animals, difficulty in the HEV sero-surveillance of this wild population because of bad quality serum samples to perform serologic tests. With this purpose, this study aimed to determine whether meat juice as an alternative sample for HEV Enzyme-Linked Immunosorbent Assay (ELISA) test is appropriate to predict the occurrence of HEV in the wild boar populations. During the 2021/2022 hunting season, 123 paired samples were obtained (blood vs meat juice) from hunted wild boars in Portugal. In the laboratory, a commercial ELISA kit was performed. To minimise the error and influence of hemolysis on serum testing, only 60 paired samples were considered, and the overall agreement proportion is 58%. With these results, this study has some limitations, and the potential use of meat juice for serologic tests performance should be discussed.

Background

Pigs have been identified as a main animal reservoir of the zoonotic Hepatitis E virus (HEV). The circulation of this virus in the wild boar population is also a risk factor for domestic pigs, especially outdoor production ones (Martinelli et al., 2015; Ruiz-Fons et al., 2017; Sanno et al., 2014). In humans, it can be responsible fan acute hepatitis, and consuming raw and undercooked pork products, that is, liver, meat or meat products, is known as a potential risk of foodborne HEV infection (Gomes-Neves et al., 2021). Wild boars are an excellent sentinel for HEV, and their sero-surveillance may allow the calculation of the potential zoonotic risk to humans (Sridhar et al., 2015). The blood collected from hunted animals is the best sample for sero-monitoring. Still, isolating sera from wild animals' blood in the field is difficult because special equipment and techniques are required. In the case of collections from hunted animals, these collections are even more complicated due to most of the time, the collected blood is hemolysed and/or degraded, and the serum obtained does not allow adequate screening laboratory tests. Meat juice is an easiest sample and can be collected by freezing and thawing of heart and diaphragm and contains antibodies against the Hepatitis E virus (Yonemitsu et al., 2019).
The aim of this study was to determine whether meat juice as an alternative sample for HEV Enzyme-Linked Immunosorbent Assay (ELISA) test is appropriate to predict the occurrence of HEV in the wild boar populations.

Materials and Methods

During the 2021/2022 hunting season, 123 paired samples were obtained (blood vs meat juice) from hunted wild boars in Portugal. All collected samples in the field were correctly accommodated, labelled, and taken to the laboratory under appropriate refrigeration conditions.

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In the laboratory, blood was centrifuged, and serum was collected and stored until use. Meat juice was obtained after approximately 100g of muscle (as diaphragm and heart) were frozen and thawed at environmental temperature, and the drained fluid was collected. Subsequently, the ELISA test was performed with the two parallel samples per the recommendations. Samples were tested for anti-HEV antibodies using a commercial double-antigen multi-species sandwich HEV ELISA kit (MP Diagnostics, Illkirch, France), following the manufacturer's instructions for serum diluting and using a concentration 10 times higher for meat juice as recommended in other published scientific papers.

Results

In the ELISA tests, separately, a prevalence of 28% was obtained for serum (34 seropositive in 123 samples) and for meat juice, the prevalence increased to 48% since 59 samples were positive for the test. With these different results with the two different matrices, the possible influence of the serum hemolysis on the negativity of the samples was calculated since, of the 123 sera collected, 63 (51%) were characterised as medium or highly hemolysed. Thus, using a statistical Fisher's exact Test, it was proved that there is a statistically significant association between the serum hemolysis and the ELISA test negativity (p-value < 0.05). And the probability of seronegativity was 0.2 times higher (OR = 0.2) in the hemolysed samples.

To minimise the error and influence of hemolysis on serum testing, to measure the agreement between the parallel assays (in sera vs meat juice), Cohen's kappa coefficient *(k)* was used. Only 60 (non-hemolysed) samples were considered, and the overall agreement proportion is 58%, with a negative agreement proportion of 70% and a positive agreement proportion of only 32%.

Discussion and conclusions

The seroprevalence of HEV in Europe has been decreasing, both in domestic and wild pigs, and its endemicity in the wild boar population of Portugal is unclear.

In the case of large game species such as wild boar, serum samples, due to the inherent difficulties involved in their collection, such as rapid degradation, hemolysis, and difficulty obtaining it, do not constitute a good sample for reliable sero-surveillance (Felin et al., 2015). Although the results of this study are not conclusive and irrefutable, in our opinion, meat juice is an excellent feasible alternative sample to serum to perform ELISA tests for HEV.

This is a sample that is easy to obtain *post-mortem*. Its use can be widespread outside the hunting world, including in domestic pig slaughterhouses, where the diaphragm is systematically collected from screening for Triquinella (Vieira-Pinto et al., 2021). Utilising this collected muscle, the obtained meat juice could be a valuable source of information on the several infectious agents' level of infection, particularly in the HEV case. Other scientific study has shown a correlation between a positive HEV serology and the occurrence of HEV RNA in the liver (Kozyra, et al., 2020) and, in our case, the paired meat juice sample with the only HEV RNA positive found out of our 123 livers analysed (data not shown), was positive in the ELISA test.

This study is limited, and the potential use of meat juice, in this case, should be discussed. The hemolysed serum, the potential meat juice's false positives and the small number of samples considered to measure the agreement score can influence these results. To reduce the *bias* of the presented study, more studies should be carried out in the future, such as other experimental methods and serial meat juice dilution tests or even the differentiated dilution by HEV endemicity according to the sample origin. The hypothesis of surveying with similar characteristics in domestic pigs, especially in extensive ones, should also be considered.

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